polymerase chain reaction and to methods of simultaneous amplification and detection of multiple DNA target sequences present in a DNA sample. The claimed invention also relates to a method for high-throughput genetic screening and to a method of screening to simultaneously detect amplification products of multiple target sequences in a DNA sample. The invention is directed, as well, to a multiplicity of amplified target DNA sequences of interest detected according to the claimed method.

The Pending Claims

Prior to entry of the above amendments, Claims 1-18 are pending. Claims 1, 2, 3, 4, 5 are directed to oligonucleotide primers. Claims 6 and 13 are directed to a method for simultaneous amplification of multiple DNA sequences by multiplex PCR. Claims 7, 8 and 9 are directed to a method for detecting multiplex PCR amplification products. Claims 10, 11 and 12 are directed to a method for high-throughput genetic screening. Claims 14, 15 and 16 are directed to a method to detect multiple amplified target DNA sequences. Claims 17 and 18 are directed to multiplex PCR amplified target sequences.

The Advisory Action

The Examiner has withdrawn the 35 U.S.C. 112 first paragraph rejection of claims 17 and 18.

The Examiner has withdrawn the 35 U.S.C. 132 objection to the amendment filed 2/10/97 in light of the amendments of the response filed 9/15/97.

The Examiner has not entered the proposed amendments to claims 1 and 14 containing the limitation "common sequence (oligonucleotide) not comprising a restriction enzyme recognition site sequence" as requiring further consideration and search and raising the issue of new matter, as support was not pointed out in the specification.

The Examiner has maintained the 35 U.S.C. 103 rejection of claims 1-5 over Weighardt *et al.* and of claims 6-12 over Picci *et al.* in view of Weighardt *et al.* Claims 13 and 17 are

likewise rejected over Picci *et al.* in view of Weighardt *et al.* However, see Telephonic Clarification below.

The 35 U.S.C § 112, second paragraph rejection of claim 17-18 remain outstanding.

Telephonic Clarification of Advisory Action

The Examiner stated in a telephone conversation on November 24, 1997, that Claims 13-18 were rejected under U.S.C. 103 and not Claims 13 and 17 as was stated in the Advisory Action of October 17,1997.

The summary page of the Advisory Action indicates that the amendments submitted September 15, 1997 were not entered, however, paragraphs 1-3 on page 2 of the Advisory Action indicate that only the proposed amendments to Claims 1 and 14 were not entered. The Examiner confirmed that the information on page 2 of the Advisory Action is incorrect.

Amendments

Claims 1, 2, 3, 4, 5, 6, 7, 10, 13, 14 have been amended and support is found at page 3, line 19; page 8, lines 7-10; page 15, lines 2-6; page 16, line 2; page 9 lines 21-22 to page 10 lines 1-2.

Claims 8 and 9 has been amended to use consistent language relating to claim dependency and to use language which has proper antecedent basis.

Claims 11 and 12 have been amended to use language which has proper antecedent basis.

Claims 15 and 16 have been amended to use language which has proper antecedent basis.

Claims 17 and 18 have been amended to recite the language from the claims from which they previously depended. Support is found at page 3, line 19.

No new matter is added by these amendments. The amendments are believed to put the case in form for allowance or in better form for appeal and the Examiner is respectfully requested to enter them.

Response

In the response that follows, the Examiner's specific objections and rejections are reiterated in small bold indented print, followed by Applicant's response, which is identified by normal print.

Section 132

The Examiner has not entered the proposed amendments to claims 1 and 14 containing the limitation "common sequence (oligonucleotide) not comprising a restriction enzyme recognition site sequence" as requiring further consideration and search and raises the issue of new matter, as support was not pointed out in the specification.

> **Proposed amended claims 1 and 14 contain the limitation "common sequence (oligonucleotide) not comprising a restriction enzyme recognition site sequence" which required further consideration and search. Further this limitation raises the issue of new matter, as support has not been pointed out in the specification. Therefore, the amendment has not been entered.**

The Examiner's comment is acknowledged.

Section 112, second paragraph

Claims 17 and 18 remain rejected because the language "a plurality of amplified target sequences of interest" because it cannot be determined from the specification what is encompassed by a plurality of nucleic acid defined only by how they are detected. This rejection is believed avoided by amendment of Claims 17 and 18 to recite the specific steps from Claim 13 and 14 that lead to the reproduction of the amplified target DNA sequences.

Section 103

The Examiner has rejected Claims 1-5 over Weighardt *et al.* This rejection is respectfully avoided in part and traversed in light of the above amendments. The Applicant's invention is patentable over the art cited by the Examiner by providing a multiplicity of single-stranded oligonucleotide DNA primers having a common 5' sequence with no homology to any multiple target DNA sequence and a unique 3' target sequence that is contained within or flanking one of the multiple target DNA sequences or its complement. In the amended claims, the 5' and 3' domains, X and Y, respectfully, are further defined as having sequences that prevent primer-primer dimer formation and the synthesis of spurious fragments.

The Examiner stated in the Office actions of 2/10/97 and 6/13/97 that Weighardt *et al.* teaches the claimed invention. The Applicants respectfully disagree. Weighardt *et al.* disclose tailed primers in which the 5'-sequence is "unrelated to the template" as containing a palindromic, restriction endonuclease recognition site (5'-ATGCAT; page 79, Figure 2; page 79, right column, lines 38-40). This type of primer used by Weighardt promotes primer-primer dimer formation and would not be functional in either a standard or single-step, multiplex PCR. In Figure 2, Weighardt *et al.* provides evidence that primers tailed with palindromic, restriction endonuclease site sequences are not functional in a single-step, standard PCR. On page 78, right column, lines 7-12, Weighardt *et al.* state: "As shown in Figure 2A, the standard PCR (with both sets of primers, normal and tailed ones) yielded very little of the expected 330-nucleotide band contaminated by a great number of spurious fragments of different size." Weighardt *et al.* reiterate the limitation of their primers on page 78, right column, lines 40-44: "As shown in Figure 3 (left), with the standard PCR protocol several smaller bands were produced by the reaction, in addition to the expected 155 nucleotide band." In these examples, "standard PCR protocol" refers to PCR without individual primer extension reactions prior to high-stringency amplification. The presence of the palindromic sequence may account for the necessity in Weighardt *et al.*'s procedure for a two-step amplification reaction involving separate primer extensions with each primer. Because the tailed primers of Weighardt *et al.* do not function in

single-step, standard PCR it can be assumed that they would not function in the single-step, multiplex PCR as disclosed by the claimed invention. Therefore, the Examiner's objection regarding "The benefit of using Weighardt *et al.*'s primers having non-complementary 5'-end tails would have been expected in the multiplex PCR method of Picci *et al.*" is also respectfully traversed because the primers of Weighardt *et al.* are non-functional in a single-step, standard PCR.

The Examiner has stated that Weighardt *et al.* teaches a sequence that provides a high stringency binding site and teach compositions that provide such sites. Weighardt *et al.* does recite "the entire primers in turn become new and unique high stringency recognition sites in the following PCR cycles" (pg.77). However, the primers disclosed by Weighardt *et al.* differ significantly in their composition from the primers disclosed in the amended claims, in that, Weighardt *et al.*'s primers contain restriction enzyme sequences (Figure 2 legend, page 79) that because of their palindromic composition would promote primer-primer dimer formation, whereas, primers of the claimed invention are designed to avoid such sequences. Therefore, the composition of Weighardt *et al.*'s primers teaches away from the claimed invention.

The Examiner has also stated in the Office action of 10/9/96 that it would have been "obvious to one of ordinary skill in the art at the time of the invention to make the primer as claimed" and that "Weighardt gives a broad teaching of any possible high stringency binding site." The Applicants respectfully disagree. Weighardt *et al.* teach primers having a 5' domain comprising a sequence unrelated to the target but, in contrast to the claimed invention, Weighardt *et al.* teach primers that have palindromic, restriction endonuclease sequences. The Applicant's respectfully disagree with the Examiner's argument that the sequence disclosed in the claimed invention represents "one of a large number of sequences expected to work." If this were true, then the primers as taught by Weighardt *et al.* would function in a single-step, multiplex PCR. However, Weighardt *et al.* state on page 79, Figure 2 legend; page 78, right column, lines 7-12; and page 78, right column, lines 40-44, that their primers do not function in a single-step, standard PCR. Therefore the primers of Weighardt *et al.* would not function in a single,

multiplex PCR. In light of Weighardt *et al.*'s teaching, it would have required extensive experimentation to arrive at the primer sequences disclosed in the claimed invention because Weighardt *et al.* taught away from the claimed invention.

The Examiner has rejected Claims 6-18 under 35 U.S.C. 103 as being unpatentable over Picci *et al.* (*Hum. Genetic.* 1992 Vol. 88, pg. 552-556) in view of Weighardt *et al.* The Examiner stated in reference to Claims 6-12:

> **One having ordinary skill in the art at the time the invention was made would have been motivated to modify the primers in the method of Picci *et al.* to contain a 5' sequence providing a high stringency binding site because Weighardt teaches the primers used in his reaction have unique high-stringency binding sites in the PCR cycle. The claimed sequence is one of the equivalents which would have been expected to work in the method. It would have been prima facie obvious to carry out the claimed methods.**

The Applicants respectfully traverse this rejection because one of ordinary skill in the art would not have succeeded in carrying out the multiplex PCR of Picci *et al.* using primers taught by Weighardt *et al.* The method of Picci *et al.* requires "the sequential addition of individual primer pairs into a single reaction" (page 553, left column) and, Picci *et al.* state that their PCR primers formed "primer-dimers" that generated unwanted, additional products (page 553, right column). These statements contrast with the examples provided in instant specification. The claimed methods use primers that prevent primer-primer dimer formation and the synthesis of spurious amplification products. The addition of Weighardt *et al.* does not cure the deficiencies of Picci *et al.*

The Examiner stated in the Office Action of 6/13/97 that "Weighardt *et al.* do suggest that the method can attain the same results in one step using a single set of primers (see page 79)." The Applicants respectfully disagree. Weighardt *et al.* do state on page 79 that, "Our method can instead attain the same result in one step and with a single set of primers." However, the definition of "one step" as used by Weighardt *et al.* differs from the claimed invention

language of single multiplex PCR. The "one-step" procedure of Weighardt *et al.* is described on page 78, right column, lines 49-61 to page 79, right column, lines 1-3. The "one step" of Weighardt *et al.* refers to the simultaneous addition of both oligonucleotides and carrying out individual extension reactions at temperature-one (T_1) and temperature-two (T_2) for each primer prior to high-stringency PCR. Weighardt *et al.* define this as one-step because both primers are added simultaneously which contrasts to their first example in which the addition of the individual primers is followed by individual extension reactions carried out at a temperature optimum for each primer. This differs from the claimed invention where individual extension reactions are not required, only high-stringency PCR. Although Weighardt *et al.* refer to the simultaneous addition of both primers followed by individual primer extensions at specific temperatures prior to high-stringency PCR as a one step procedure, it is in actuality a two-step PCR because of the requirement for individual extension reactions for each primer (Weighardt *et al.* page 77, center column, lines 60-61 to page 77, right column, lines 1-5). On page 79, Figure 2 legend; page 78, right column, lines 7-12; and page 78, right column, lines 40-44, Weighardt *et al.* state that their primers do not function in a single-step, standard PCR. Therefore the primers of Weighardt *et al.* would not function in a single, multiplex PCR as described in the claimed invention. Moreover, Picci *et al.* state that their primers form "primer-dimers" and additional bands (page 553, right column). Therefore, if one used primers containing tailed sequences as taught by Weighardt *et al.*, primer-dimer formation and the appearance of additional bands would have been increased because Weighardt *et al.* taught primers having palindromic restriction enzyme sequences that promote primer-dimer formation. To arrive at the compositions and methods of the claimed invention would have required extensive experimentation.

Weighardt *et al.* do not teach methods for the simultaneous amplification of multiple target DNA sequences employing single multiplex PCR. Weighardt *et al.* teach a two-step PCR method for the amplification of a single-target. The Examiner's comment that the benefit of using Weighardt *et al.*'s primers having non-complementary 5'-end tails would have been expected in the multiplex PCR method of Picci *et al.* is also respectfully traversed. As stated

above, on page 78, right column, lines 7-12 and on page 78, right column, lines 40-44 Weighardt *et al.* state that their primers do not function in a single-step standard PCR and therefore it can be concluded that they would not function in a single multiplex PCR.

The claimed invention discloses primers and methods for single multiplex PCR and methods for the detection of the PCR amplification products. The claimed primers are designed to prevent primer-primer hybridization and the formation of spurious amplification products. Weighardt *et al.* state on page 78, right column, lines 7-12 and on page 78, right column, lines 40-44 that their primers do not function in a single-step standard PCR and therefore it can be concluded that they would not function in a single multiplex PCR. Weighardt *et al.* do not claim that their primers will function in a multiplex PCR under any conditions.

In summary, the claimed invention is patentable over the references cited by the Examiner because it overcomes the technical limitations of the prior art in providing primers and methods for the amplification and detection of multiple target DNA sequences in a single, multiplex PCR. Accordingly, the rejection of Claims 1-5 and 6-18 under 35 U.S.C. § 103 should be withdrawn.

CONCLUSION

In view of the above amendments and remarks, it is submitted that this application is now ready for allowance. Early notice to that effect is solicited. If in the opinion of the Examiner, a telephone conference would expedite the prosecution of the subject application, the Examiner is invited to call the undersigned at (650) 328-4400.

Respectfully submitted,

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